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P/2850-106

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Confirmation No.: 2037

Shigeyuki YOKOYAMA, *et al.* Date: December 4, 2008

Serial No.: 10/532,948 Group Art Unit: 1656

Filed: January 9, 2006 Examiner: Kagnew H. Gebreyesus

For: **METHOD OF EXPRESSING PROTEINS COMPRISING NON-NATURALLY OCCURRING AMINO ACIDS**

VIA EFS –WEB
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT UNDER 37 C.F.R. § 1.116

Sir:

This is a response to the final Office Action mailed August 7, 2008 in the above-identified application. Reconsideration of the application is respectfully requested.

FEE CALCULATION

Any additional fee required has been calculated as follows:

____ If checked, "Small Entity" status is claimed.

	No. Claims After Amendment		Highest No. Previously Paid For		Extra Present		Rate	ADDIT. FEE
TOTAL	12	MINUS	20	* =	0	X	($26 SE or $52)	$ 0
INDEP	4	MINUS	4	** =	0	X	($110 SE or $220)	$ 0
First Presentation of Multiple Dependent Claim						X	($190 SE or $380)	$
* not less than 20 ** not less than 3							TOTAL	$ 0

A Petition for Extension of Time, along with the proper corresponding fees, is submitted with this Amendment. In accordance with 37 C.F.R. § 1.136(a), a fee for filing a Petition for Extension of Time for one (1) month, from November 7, 2008 to and including December 7, 2008, of $130, pursuant to 37 C.F.R. §1.17(a)(1) is also submitted. Credit card payment in the amount of $130.00 is submitted via EFS-Web to cover the calculated fee.

In the event the actual fee is greater than the payment submitted or is inadvertently not enclosed or if any additional fee during the prosecution of this application is not paid, the Patent Office is authorized to charge the underpayment to Deposit Account No. 15-0700.

CONTINGENT EXTENSION REQUEST

If this communication is filed after the shortened statutory time period had elapsed and no separate Petition is enclosed, the Commissioner of Patents and Trademarks is petitioned, under 37 C.F.R. § 1.136(a), to extend the time for filing a response to the outstanding Office Action by the number of months which will avoid abandonment under 37 C.F.R. § 1.135. The fee under 37 C.F.R. § 1.17 should be charged to our Deposit Account No. 15-0700.

SUMMARY OF AMENDMENTS

1. ___ If checked, an abstract (an amended abstract) is submitted herewith.

2. ___ If checked, amendment(s) to the drawings are submitted herewith.

3. ___ If checked, amendment(s) to the specification are submitted herewith.

4. _X_ If checked, amendment(s) to the claims are submitted herewith.

LISTING OF THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claim 1 (currently amended): An expression method for non-naturally-occurring amino acid-containing proteins comprising: expressing in isolated animal cells:

(A) a mutant tyrosyl-tRNA synthetase (V37C195) that has undergone alterations from tyrosine to valine at position 37 and from glutamine to cystein at position 195 of tyrosyl tRNA synthetase originating from *E. coli.* (SEQ ID NO:29) with an enhanced specificity for a non-naturally- occurring tyrosine derivative selected from the group consisting of 3-iodotyrosine and 3-bromotyrosine, as compared with the specificity for tyrosine;

(B) *Bacillus stearothermophilus* suppressor tRNA ~~originating in *Bacillus stearothermophilus*~~ capable of binding with the tyrosine derivative in the presence of the mutant tyrosyl tRNA synthetase; and,

(C) a desired protein gene that has undergone a nonsense mutation at a desired site; wherein, the tyrosine derivative is incorporated in response to the created nonsense codon.

Claims 2-5 (canceled).

Claim 6 (previously presented): The expression method according to claim 1 wherein the isolated animal cells are mammalian cells.

Claim 7 (previously presented): A non-naturally-occurring amino acid-containing protein production method comprising: recovering and purifying a protein expressed in an isolated animal cell according to the method according to claim 1.

Claim 8 (withdrawn): Animal cells containing:

(A) an expression vector that expresses in animal cells a mutant tyrosyl-tRNA synthetase that is a derivative of tyrosyl tRNA synthetase from *E. coli* with an enhanced specificity for a non-naturally-occurring tyrosine derivative as compared with the specificity for tyrosine;

(B) an expression vector that expresses in the animal cells a suppressor tRNA originating in a Bacillus species, Mycoplasma species or Staphylococcus species of eubacteria capable of binding with the tyrosine derivative in the presence of the mutant tyrosyl tRNA synthetase; and,

(C) an expression vector that expresses in the animal cells a desired protein gene that has undergone a nonsense mutation at a desired site; wherein,
the tyrosine derivative is incorporated at the site of the nonsense mutation of the protein.

Claim 9 (withdrawn): The animal cells according to claim 8 wherein the tyrosine derivative is a position 3-substituted tyrosine or position 4-substitued tyrosine.

Claim 10 (withdrawn): The animal cells according to claim 8 wherein the suppressor tRNA of (B) is suppressor tyrosine tRNA originating in *Bacillus stearothermophilus*.

Claim 11 (withdrawn): The animal cells according to claim 8 wherein the mutant tyrosyl tRNA synthetase of (A) is a mutant tyrosyl tRNA synthetase that has undergone an alteration at the location corresponding to tyrosine at position 37 and glutamine at position 195 of tyrosyl tRNA synthetase.

Claim 12 (withdrawn): The animal cells according to claim 11 wherein the mutant tyrosyl tRNA synthetase of (A) is a mutant TyrRS in which the location corresponding to tyrosine (Y) at position 37 of tyrosyl tRNA synthetase is substituted with valine (V), leucine (L), isoleucine (I) or alanine (A), and the location corresponding to glutamine (Q) at position 195 of tyrosyl tRNA synthetase is substituted with alanine (A), cysteine (C), serine (S) or asparagine (N).

Claim 13 (withdrawn): The animal cells according to claim 8 that are mammalian cells.

Claim 14 (withdrawn): DNA having a sequence selected from the group consisting of SEQ. ID NO. 1, SEQ. ID NO. 30, SEQ. ID NO. 31 and SEQ. ID NO. 32.

Claim 15 (withdrawn): An expression vector capable of being expressed from a control sequence recognized in animal cells comprising a sequence selected from the group consisting of SEQ. ID NO. 1, SEQ. ID NO. 30, SEQ. ID NO. 31 and SEQ. ID NO. 32.

Claim 16 (withdrawn): The expression vector according to claim 15 that carries nine copies of DNAs having the sequence of SEQ. ID NO. 1 in the same direction.

REMARKS/ARGUMENTS

Claims 1, 6 and 7 were previously pending in the present application. Claims 8-16 were previously withdrawn. Claim 1 has been amended. No new matter has been added. Entry of the current amendment to claim 1 into the file of the present application is respectfully requested as it is believed to place the entire application in condition for an allowance or, at a minimum, to materially reduce the issues for an appeal.

Applicants acknowledge that the previous rejection under 35 U.S.C. § 101 has been withdrawn by the Examiner. Applicants further acknowledge and appreciate that the prior rejection under 35 U.S.C. § 112 has also been withdrawn by the Examiner in light of the previous response. Applicants address the Examiner's final remaining grounds for rejection below.

Claim Rejections Under 35 U.S.C. §103 (Non-Obviousness Requirement)

Claims 1, 6 and 7, directed to a system for incorporating unnatural amino acids into proteins in Eukaryotic translation in isolated animal host cells, have been rejected under 35 U.S.C. § 103(a) over Kiga et al.

In response to this rejection, previous claim 1 has been amended, without prejudice or disclaimer, to clarify that which Applicants deem to be the patentable subject matter of the present application. Claim 1 has been amended to clarify that the suppressor tRNA of the invention is a *B. stearothermophilus* suppressor tRNA and not intended to encompass *B. stearothermophilus* tRNA source sequences that have been manipulated to such a degree as to be identical, in fact, with suppressor tRNA sequences of *E. coli*, a profoundly distinct species (a species distant enough in sequence so as to be readily distinguishable and not subject to rendering "identical" through only very limited amino acid residue substitutions or other sequence modifications).

Applicants further note that as the tRNAs of the *B. Stearothermophilus* and *E. coli* species have diverged in their respective sequences through natural selection, so have the sequences of the corresponding suppressor tRNA and aminoacyl tRNA synthetases for each species (e.g., the native *E. coli* tyrosyl-tRNA synthetase sequence has but 56% identity to that of *B. stearothermophilus*). Thus, a scientist practicing in the field would understand that the sequence of the tRNA and the tRNA synthetase for each independent species would likely

coevolve together over time, but the same scientist would have no expectation that there would be any convergence between the sequences specific to the *B. stearothermophilus* suppressor tRNA (or tRNA synthetase) and those of the *E. coli* suppressor tRNA (or tRNA synthetase). Nor would such a scientist presume or reasonably expect an interchangeability or compatibility in functioning between the *B. stearothermophilus* suppressor tRNA with that of *E. Coli* suppressor tRNA, particularly with regard to association with the *E. coli* mutant tyrosyl-tRNA synthetase.

Recognizing the distinct sequences and properties of the *B. stearothermophilus* and *E. coli* suppressor tRNAs, respectively, and having no reasonable expectation that the suppressor tRNAs between species would be functionally compatible or equivalent, a scientist of average skill in the art would have no motivation to use a combination across these species, particularly a combination of the tRNA of *B. stearothermophilus* with the mutant tyrosyl-tRNA synthetase from *E. coli*, even in view of the Kiga reference teachings.

This lack of motivation is reinforced by the fact that *E. coli* tRNA synthetase coupled with *E. coli* suppressor tRNA (in both wild type and mutant form), when expressed in animal cells as per the present invention, failed to demonstrate suppression (and thus failed to perform the methods of the present invention). *See, e.g.*, pp. 48-51 and FIG. 4 of the present specification. Thus, even using the *E. coli* tRNA synthetase as an initial starting point for the present methods, let alone to combine the *E. coli* tRNA synthetase across species with a *B. stearothermophilus* suppressor tRNA, would be insufficiently motivated.

To the contrary, it is only by relying upon the disclosure contained in the present application that a scientist practicing in the field would recognize the value of and be motivated to practice the current methods as recited in claim 1. This holds true even as the present disclosure demonstrates suppression activity by the *B. stearothermophilus* suppressor tRNA associated with *E. coli* tRNA synthetase expressed in animal cells without alteration. While such cross-species findings are useful and are, in fact, important to the present invention, they were not readily predictable or to be reasonably expected prior to the teachings of the present disclosure.

Claim 1 as recited, along with pending claims 6 and 7 dependent thereto, is now believed to allay the concerns set forth by the examiner in the Office Action and to overcome the rejection under 35 U.S.C. § 103(a). Applicants respectfully ask that the examiner reconsider the

outstanding rejection under 35 U.S.C. § 103(a), in view of the amendments made to claim 1, and to deem that claims 1, 6 and 7 to be in suitable condition for prompt allowance.

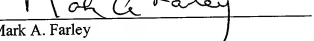
Summary

This Amendment is believed to overcome all of the grounds for objection and rejection set forth in the August 7, 2008 Office Action regarding this application, which should therefore be withdrawn.

If the Examiner does not agree, however, but believes that an interview would advance the prosecution of this case, the Examiner is respectfully invited to telephone applicants' representative at the number below in order that an interview concerning this application may be scheduled.

Respectfully submitted,

THIS CORRESPONDENCE IS BEING SUBMITTED ELECTRONICALLY THROUGH THE PATENT AND TRADEMARK OFFICE EFS FILING SYSTEM ON December 4, 2008



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